

Gross anatomy and positional homology of gills, gonopores, and nephridiopores in “basal” living chitons (Polyplacophora: Lepidopleurina)*

Julia D. Sigwart

National Museum of Ireland, Natural History Division, Merrion Street, Dublin 2, Ireland and Queen's University Belfast, School of Biological Sciences, University Road, Belfast, BT7 1NN, Northern Ireland, UK, julia.sigwart@ucd.ie

Abstract: Traditional shell characters are insufficient to differentiate taxa within the polyplacophoran order Lepidopleurida. Additional morphological character sets from soft anatomy (e.g., gamete morphology, gill arrangement, and locations of gonopores and nephridiopores) have previously been described from only a small number of taxa. This study reports for the first time, positions of the gonopores and nephridiopores for 17 species in the Lepidopleurina. The position of both types of pores on the longitudinal body axis varies within a generalized range of the posterior third of the body; however, the separation between the pores as a proportion of the specimen's foot length varies from 3.7% to 17% in different species. Positions of pores relative to the serial gills are also variable within species, and future studies may require a new descriptive basis in order to resolve positional homology. The order Lepidopleurida occupies a critical position with respect to understanding larger-scale patterns in polyplacophoran (and molluscan) evolution.

Key words: *Leptochiton*, Leptochitonidae, descriptive morphology, molluscan evolution

Recent polyplacophorans are divided into two orders: the large, diverse order Chitonida, and the putatively basal Lepidopleurida. All living taxa in this order are grouped into five families within the suborder Lepidopleurina (Sirenko 2006). The group contains around 120 living species in nine genera, although more than eighty of these taxa are traditionally classified in the single genus *Leptochiton* Gray, 1847. All species in Lepidopleurina are characteristically small, without shell insertion plates, rarely with complex shell sculpture or girdle elements, and often found in deep sea habitats including hydrothermal vents (Saito and Okutani 1990), sunken-wood deposits (Sirenko 1988, 1997), and cold-seeps (Kiel and Little 2006). The predominant characteristics of this group could be that they live in the most inaccessible habitats frequented by chitons and are the most difficult to examine once captured.

Detailed understanding of these chitons has progressed slowly; the animals are fundamentally difficult to identify because of their small, plain appearance, and inconsistent nomenclature remains a major source of confusion. For clarity, members of the suborder Lepidopleurina are collectively referred to as “lepidopleurans”. The two genus names “*Lepidopleurus*” and “*Leptochiton*” were historically often used interchangeably. There remains persistent confusion over the family name Leptochitonidae Dall, 1889 and its proposed replacement Lepidopleuridae Pilsbry, 1892 (Dall 1889,

Pilsbry 1892, Dell'Angelo and Palazzi 1991). Despite the nomenclatural irregularity with the corresponding subordinal and ordinal epithets, the family name Leptochitonidae has clear priority and is the taxonomically correct name for the group.

Although classical descriptions rely on shell characters (or lack thereof), lepidopleurans are also universally allied by their posteriorly arranged gills and apparently simple gametes. Cladistic studies of Polyplacophora have repeatedly recovered the major subordinal groups, with Lepidopleurina as the sister group to other living chitons (Buckland-Nicks 1995, Okusu *et al.* 2003). These studies have also shown that gamete morphology corresponds strongly to these major taxonomic partitions. This corroborates Sirenko's (1993) work correlating gamete structures to gill arrangements for more than 100 species; although Sirenko (1993) reported the position of nephridiopores and gonopores for 130 species in the Chitonida, he included no lepidopleurans.

To date, the gamete morphology for seven species of lepidopleuran chiton have been published: *Leptochiton asellus* (Gmelin, 1791), *Leptochiton rugatus* (Carpenter MS, Pilsbry, 1892), *Leptochiton assimilis* (Theile, 1909), *Deshayesiella curvata* (Carpenter MS, Pilsbry, 1892), *Hanleya hanleyi* (Bean in Thorpe, 1844), *Hanleyella asiatica* Sirenko, 1973 (Sirenko 1993, Hodgson *et al.* 1988, Buckland-Nicks 2006). Of those, most have smooth egg hulls, but *Hanleyella asiatica* has long

* From the symposium “Advances in Chiton Research” presented at the joint meeting of the American Malacological Society and Western Society of Malacologists, held 29 July to 3 August 2006 in Seattle, Washington.

hair-like chorion processes on the egg hull, similar to other (unrelated) brooding species. However, work on other brooding species with reduced chorion processes indicates that egg hulls show little homoplasy (Sirenko 1993, Eernisse and Reynolds 1994). The lepidopleuran sperm type is also considered plesiomorphic as it lacks the relatively complex acrosome processes characteristic of all described sperm from non-lepidopleuran chitons (Buckland-Nicks 2006).

Gamete characteristics are of great interest, but examination is limited in a practical way by fixation and specimen availability. The gross morphology of the exterior position of the gonopore, which can be examined on preserved specimens, may also be related to fertilization and reproductive biology. The gonopore and nephridiopore in all chitons are located on the roof of the pallial cavity between the gills (Fig. 1).

More than 50% of lepidopleuran taxa are found at a minimum depth of at least 100 m, and many recently described species in the Leptochitonidae have a maximum

adult length of 5 mm or less. However, it is still not understood how many morphological features may respond to environmental conditions within the lifespan of the individual animal.

This paper presents a series of preliminary descriptions on the position, number, and distribution of gills, gonopores, and nephridiopores across a sample of basal living chitons. Little previous work of this type has been conducted, especially in these very small animals; it is hoped that a survey of this kind will initiate additional morphological (especially microanatomical) and phylogenetic studies within Lepidopleurina. There is little doubt that this clade of chitons will prove of critical importance to our understanding of polyplacophoran evolution.

MATERIALS AND METHODS

This study included examination of the alcohol-preserved material in the former collection of P. Kaas (1915-1996) held in Naturalis, Leiden, The Netherlands (RMNH) and that of R. A. Van Belle (1920-2005) in the Royal Belgian Institute of Natural Sciences, Brussels, Belgium (RBINS). These two collections make up a substantial proportion of the fluid-preserved polyplacophoran material in their respective institutions. The majority of specimens were historically fixed in formalin before being transferred to methylated ethyl alcohol (70%); some are presumed to have been additionally preserved with glycerin. Both collections include material that was collected by Kaas and Van Belle as well as other colleagues who contributed material to their private collections. The specimens vary in collecting age from the 1970s to the 1990s. As the majority of specimens were collected by persons knowledgeable about chitons and were flattened at preservation, they are in excellent condition for morphological examination.

All specimens that were preserved in a flattened posture were examined under a dissecting microscope (up to 100× power) for observing the number of gills per side. The location of the gonopores and nephridiopores relative to gills was determined without dissection, usually by using 000 gauge insect pins as a probe to separate gills for inspection of the pallial cavity roof. Use of flexible insect forceps allowed the specimen to be held in place without damage and while immersed in fluid. In some cases for specimens in RBINS, a small strip of tissue was removed from the lateral margin of the foot at the posterior end, to facilitate viewing the gill row. In other cases for specimens in RBINS and RMNH, individual gills were removed ("pinched" off) to facilitate viewing the pallial cavity roof. Multiple specimens in a species were examined where suitable material was available.

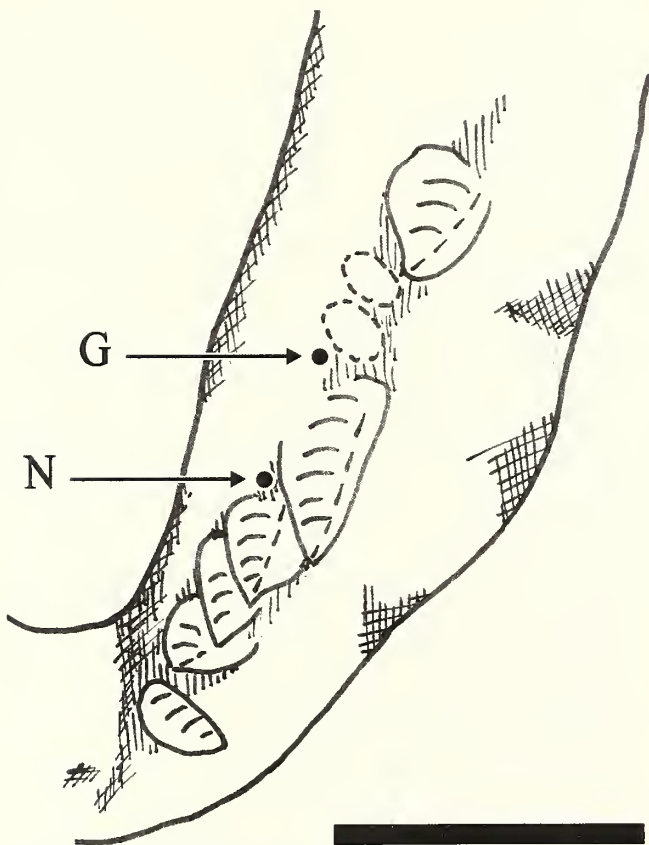


Figure 1. Camera lucida drawing of *Leptochiton norfolcensis* (Hedley and Hull, 1912); scale bar = 5 mm. Note nephridiopore (posterior) between gill 4 and 5, and gonopore between gill 5 and 6 (gill 6 and 7 removed, indicated by dashed outline).

Position of nephridiopores and gonopores was recorded relative to the posterior end of the gill row. Gills were numbered from the one closest to the anus and counted in series, following Sirenko (1993). Positions of the pores were recorded as between the two closest gills, or in some cases, one gill when the pore was directly in front of the base of a single gill.

Measurements to the nearest 0.1 mm were taken with calipers for total body length, foot length, and length of the gill row. As the number of gills may vary between left and right sides of individual specimens, all pore placements were confirmed on both sides; gill counts and measurements were taken uniformly from the left side of the animal. These measurements were omitted where specimens were damaged or too curled to allow accurate measure. Rapid degradation of the external gill tissue after death means that only the best-preserved specimens were suitable for determination of the location of the gonopores and nephridiopores by this technique. The separation between gonopore and nephridiopore was estimated based on the number of gills separating pore locations and the average inter-gill distance (length of row divided by number of gills per side). This is possible with some degree of accuracy, as pores are consistently placed in the middle portion of the gill row, where each individual gill is of "average" width. All measurements reported were taken from a mean of three successive caliper measures.

RESULTS

A total of 49 species from eight lepidopleuran genera were examined for placement of gonopore and nephridiopore; however, pore locations relative to the gills were successfully determined in only 17 species (Table 1). The specimens ranged from 4.7 mm to 20.6 mm in body length. Specimens had between 4 to 21 gills per side, and the gill row ranged in length from 24% to 68% of foot length (but the

Table 1. Location of nephridiopore relative to closest gills (counted from posterior) and number of gills per side in specimen.

Species	Nephridiopore	Gonopore	Gills
<i>Leptochiton algesirensis</i> (Capellini, 1859)	5-6	8-9	15
<i>Leptochiton algesirensis</i>	5-6	9-10	16
<i>Leptochiton algesirensis</i>	6-7	9-10	16
<i>Leptochiton algesirensis</i>	7-8	10-11	16
<i>Leptochiton algesirensis</i>	7-8	11-12	16
<i>Leptochiton asellus</i> (Gmelin, 1791)	5-6	7-8	10
<i>Leptochiton asellus</i>	5-6	7-8	10
<i>Leptochiton asellus</i>	5-6	7-8	10
<i>Leptochiton badius</i> (Hedley and Hull, 1908)	4-5	5-6	8
<i>Leptochiton belknapii</i> Dall, 1878	5-6	7-8	14
<i>Leptochiton cancellatus</i> (Sowerby, 1840)	5-5	6-7	8
<i>Leptochiton cancellatus</i>	5-6	6-7	8
<i>Leptochiton cancellatus</i>	5-6	6-7	8
<i>Leptochiton cancellatus</i>	5-6	7-7	8
<i>Leptochiton cancellatus</i>	5-6	6-7	8
<i>Leptochiton foresti</i> (Leloup, 1981)	7-8	8-9	10
<i>Leptochiton kurnilatus</i> Kaas, 1985	3-4		6
<i>Leptochiton micropustulosus</i> Kaas, 1994	9	10-11	14
<i>Leptochiton micropustulosus</i>	9	10-11	14
<i>Leptochiton micropustulosus</i>	9-10	12-13	14
<i>Leptochiton norfolcensis</i> (Hedley and Hull, 1912)	4-5	5-6	8
<i>Leptochiton norfolcensis</i>	4-5	5-6	8
<i>Leptochiton rugatus</i> (Carpenter in Pilsbry, 1892)	5-6	7-7	10
<i>Leptochiton rugatus</i>	5-6	6-7	10
<i>Leptochiton sarsi</i> Kaas, 1981	5-6	7-8	9
<i>Lepidopleurus cajetanus</i> (Poli, 1791)	7	9-9	14
<i>Lepidopleurus cajetanus</i>	7-8	8-9	14
<i>Parachiton eugenei</i> (Kaas and Van Belle, 1985)	7-8	10	16
<i>Parachiton hylkiae</i> Strack, 1993	7-8	11-12	16
<i>Parachiton hylkiae</i>	8-9	10-11	16
<i>Parachiton politus</i> Saito, 1996	10-11	12-13	16
<i>Nierstraszella lineata</i> (Nierstrasz, 1905)	9-10	10-11	14
<i>Nierstraszella lineata</i>	9-10	10-11	14
<i>Nierstraszella lineata</i>	8-9	13-14	16
<i>Nierstraszella lineata</i>	8-9	13-14	16
<i>Nierstraszella philippina</i> (Leloup, 1981)	5-6	7-8	12

median value of 40% of foot length was characteristic of the suborder).

Taxa for which pore locations were successfully determined include the genera *Leptochiton* (11 species), *Lepidopleurus* (one species), *Parachiton* Thiele, 1909 (three species), and *Nierstraszella* Sirenko, 1992 (two species), represented by a total of 36 specimens. These specimens ranged in size from 5.1 mm to 15.1 mm (Table 2) and had between 6 to 16 gills per side (Table 1). The general range of placement of the gonopore and nephridiopore as a pair was variable throughout all taxa (Fig. 2). Although the two orifices have no physiological relationship, their placement and relationship with

Table 2. Size of chiton specimens; total body length measured on dorsal side in flattened position; foot length measured ventrally from mouth to anus; gill row measured on right-hand side from anus or posterior-most gill (ctenidium) to anterior-most gill.

Species	Body length (mm)	Foot length (mm)	Gill row length (mm)	Interpore distance (mm)
<i>Leptochiton algesirens</i> (Capellini, 1859)	8	5.1	2.1	0.39
<i>Leptochiton algesirens</i>	13.5	10	4.6	0.86
<i>Leptochiton asellus</i> (Gmelin, 1791)	7.8	5	1.9	0.38
<i>Leptochiton badius</i> (Hedley and Hull, 1908)	6	4.2	1.3	0.16
<i>Leptochiton cancellatus</i> (Sowerby, 1840)	5.9	3.7	1.1	0.14
<i>Leptochiton cancellatus</i>	7.1	4.7	1.5	0.19
<i>Leptochiton foresti</i> (Leloup, 1981)	6.5	4	2	0.20
<i>Leptochiton micropustulosus</i> Kaas, 1994	12.5	8.8	3.5	0.50
<i>Leptochiton micropustulosus</i>	14.6	9.2	4	0.57
<i>Leptochiton micropustulosus</i>	15.1	10.2	3.6	0.77
<i>Leptochiton norfolcensis</i> (Hedley and Hull, 1912)	5.1	3.1	1.1	0.14
<i>Leptochiton rugatus</i> (Carpenter in Pilsbry, 1892)	7.9	5.6	2	0.30
<i>Lepidopleurus cajetanus</i> (Poli, 1791)	11.5	8.5	3	0.43
<i>Parachiton eugenei</i> (Kaas and Van Belle, 1985)	6.8	5	2	0.31
<i>Parachiton hylkiae</i> Strack, 1993	12.5	7.3	5.0	1.25
<i>Parachiton hylkiae</i>	9.8	6.3	3.7	0.46
<i>Parachiton politus</i> Saito, 1996	10.4	7	3.7	0.46
<i>Nierstraszella lineata</i> (Nierstrasz, 1905)	13.9	9.1	5	1.56
<i>Nierstraszella lineata</i>	13.9	9.1	5	1.56

the respiratory cavity allow us to consider them collectively for convenience here.

The placement of nephridiopores and gonopores relative to the posterior-most gill was variable among species and, in some cases, among presumed conspecifics. The majority of species in all genera (11 of 17) had a nephridiopore at the sixth or seventh gill (counted from posterior of the gill row). Other species with more posterior pores (relative to the gill row) had fewer gills—six or eight per side. However, species with more anterior pores were not correlated to higher gill counts. Although most species retained consistent pore positions in multiple specimens, some (*Leptochiton algesirens*, *Nierstraszella lineata*) varied between individuals.

The separation between nephridiopore and gonopore was also highly variable. As an estimated proportion of the length of the foot, the gap varied between 3.7% to 17% with the widest separation in *Nierstraszella lineata* and one specimen of *Parachiton hylkiae* (Table 2). This was radically different from the other specimen of *Parachiton hylkiae* for which data was available (with an interpore separation of only 7.3% of the foot length) and *Parachiton politus* (6.6%). The relationships of these proportions were similar whether considered relative to the gill row or total body length.

All species examined had gills of the adanal condition (*sensu* Sirenko 1993), having multiple gill pairs posterior of the nephridiopore. Some *Leptochiton* species would be considered “abanal” (*sensu* Plate 1899) in that all gills were of

equal length; however, these taxa were adanal (*sensu* Sirenko) as are all lepidopleurans.

DISCUSSION

The locations of gonopores and nephridiopores were considerably variable with respect to gill placements in lepidopleurans, which concurs with variability that has been reported for other chitons (Sirenko 1993). The position of the gonopore in the taxa examined was not fixed, neither positionally relative to the gill row nor in distance to the nephridiopore. This variability must be constrained by the internal organ systems but external variability is indicative of internal variability. The change in pore placement and the effects on respiration, reproduction, and excretion may mark physiological differences that impact the daily life of the animals. Variations in pore placement do not appear to be consistent with currently recognized genera, as they differ within as well as among genera.

Sirenko (1997: 13) reported pore locations for nine lepidopleuran taxa (but did not comment on these results in the discussion of his paper). None of these taxa were duplicated by this survey. However, Sirenko (1997) included two species of *Parachiton*, both of which had more than 20 gill pairs and found nephridiopore (11-12) and gonopore (12-13)

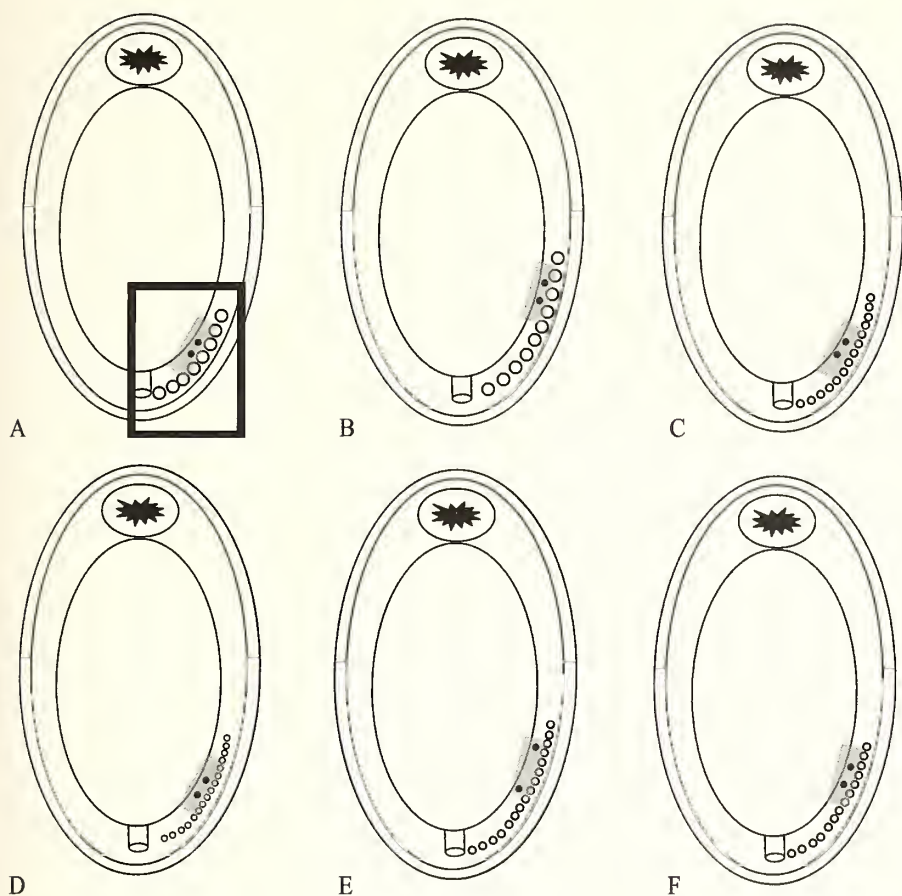


Figure 2. Schematic drawing of chiton ventral surfaces and the placements of gonopores (anterior) and nephridiopores (posterior). Drawings are stylized to emphasize positions of gills relative to gills only; the position of the nephridiopore is consistently underneath valve VII of the animal. A, *Leptochiton norfolcensis*, inset area illustrated in Fig. 1; B, *Leptochiton foresti* (Leloup, 1981); C, *Lepidopleurus cajetanus* (Poli, 1791); D, *Parachiton eugenei* (Kaas and Van Belle, 1985); E, *Nierstraszella lineata* (Nierstrasz, 1905); F, *N. lineata*.

placements that differ from the species examined in this study.

Within this morphological survey, species showed different patterns of variability. For instance, most specimens examined of *Leptochiton algesirensis* had the same number of gills per side, yet there are four different pore arrangements associated with 16 gills. This contrasts with the other most sampled species, *Leptochiton asellus*, which had extremely consistent pore positions across all specimens. In other cases there may be morphologies associated with growth patterns (or potentially cryptic species): in *Nierstraszella lineata* there is one positional morphology associated with 14 gills and another quite distinct pore arrangement in specimens with 16 gills.

The polyplacophoran nephridiopore is consistently located underneath valve VII, and the gonopore is anterior to

the nephridiopore. Non-lepidopleurans have a substantial gap between the anus and the gills, and the nephridiopore is at the posterior end of the gill row (between 1 and 2; Sirenko 1993). In lepidopleurans, however, the nephridiopore may be central within or even anterior to the gill row. This does not necessarily represent anatomical change in the pore, but rather shift in the gill arrangement morphology. Although constrained to the region under valve VII, the nephridiopore clearly varies in position relative to the body as well, as illustrated by considering the placement relative to the proportional measurement of the foot length. This should not be a surprise; for example, *Parachiton* has a characteristic enlarged tail valve, which takes up a larger proportion of the animal's foot length than in other lepidopleurans. The nephridiopore in *Parachiton* is underneath valve VII, but the nephridiopore is necessarily more anterior relative to the foot (and potentially other organ systems) than in other genera.

The variability of gill arrangements in chitons was first described by Plate (1899) by differentiating extent of the gill row (all lepidopleurans are approximately "merobranchial" with posteriorly arranged gills), presence of

small gills posterior of the major gill row, and the presence of a gap between the posterior-most gill and the anus. Ontogenetically, the adanal arrangement means that new gills are added to both ends of the gill row during growth, whereas abanal gill rows grow in an anterior direction only (with the oldest and usually longest gills at the posterior). Contemporary 19th century work proposed that the "adanal" state characterized by extra small gills at the posterior end of the row, typical of lepidopleurans, represents the plesiomorphic condition (Pelseneer 1899).

Sirenko (1993) improved Plate's (1899) classical definitions of "abanal" and "adanal" gill arrangements by reinterpreting them with reference to the nephridiopore. This usage effectively segregates the lepidopleuran taxa as an adanal clade. In the majority of lepidopleuran species, the posterior-most gills are small and sometimes bud-like processes near or attached to the anus. These minute gills are visible on

individual animals of adult size. Ontogenetically, in some species, these may grow to determinant size, but they may also be influenced by environmental plasticity, growing to suit local respiratory requirements. Kaas and Van Belle (1985) noted that counts of gills were variable in adult specimens of some species, and that counts of gills on right and left sides may vary within a single individual. Observationally, it appears that comparisons within taxa are not negatively affected as multiple individuals of the taxa included in this survey and others do have a consistent number of gills although the marginal gills may be very different in size. These structures illustrate that gills within a gill row on an individual chiton are not equal and interchangeable units.

Sirenko (1993) proposed using the posterior-most gill as a reference point for describing the location of nephridiopores and gonopores, as followed in this survey. In abanal chitons, the gill row grows in the anterior direction, making posterior-based counting a consistent method regardless of the animal's growth state. As adanal gill arrangement is presumed indicative of bi-directional growth of the gill row, using the posterior-most gill as a reference point may not be sufficient in these taxa. Examination of a single specimen may not give a positional result that can be validated in an older individual. The question of ontogeny is not fully resolved; gill row growth may be bi-directional but examination of juvenile specimens shows that the primary direction of growth is still anterior. The gill "buds" at the posterior end of the gill row appear early in post-settlement individuals. These buds are interpreted to be new gills that have not achieved full length; however, they appear in many adult sized specimens which brings into question whether some species have determinate growth in gill numbers. In examination of the gill row in a chiton of indeterminate age (as they all are), there is also no way to determine which individual gill of full size marks the "beginning" point of growth.

A number of South Pacific lepidopleurans, primarily those described by Sirenko (2001) and other related taxa, have only four gills. (Interestingly, these species would be considered "abanal" *sensu* Plate because all four gills are equal in length, but there is no question that the species are lepidopleurans). Because the entirety of the gill row is located underneath the tail valve, both the nephridiopore and gonopore are probably located on the naked pallial roof. This is a clear limitation on the present methods for describing the anatomy of nephridiopores and gonopores externally; there is no practical way to describe their placement or compare placements among these species.

Correlation of gonopores and nephridiopores, as well as internal anatomical structures, to shell morphology requires further descriptive work. Measurement of position in this case and in other reports is recorded relative to gills, but it should additionally be measured relative to dorsal valve and

sutural positions. Some of the variability observed in this morphological survey is probably an artifact of bi-directional gill row ontogeny. However, there is clearly a high level of plasticity that cannot be completely accounted for (particularly in the well-sampled case of *Leptochiton algesirensis*) without acknowledging that the pores, and therefore internal organ anatomy, are variable within and between taxa to a higher degree than previously recognized.

In this group, as in many groups of invertebrates where species identification is challenging, new characters that help differentiate taxa are always welcome. However, the problems illustrated here demonstrate that either pore placement relative to gill elements is not positionally homologous or, alternatively, if pore placements are apomorphic then current genera are not monophyletic assemblages. Variability in this and other soft anatomical features in lepidopleurans may mask cryptic species not identifiable by traditional shell and girdle characters.

The inconsistent morphology within recognized genera in the preliminary results reported in this paper suggest that these structures may not be positionally homologous. Potential plasticity of elements in the gill row in lepidopleuran chitons is not sufficiently known and means that the positions of pores with respect to gills may be homoplasious for phylogenetic inference. It will be interesting to compare the results presented here with future hypotheses about internal relationships within the lepidopleuran clade.

Although these chitons are superficially similar, this ancient lineage of molluscs shows a great deal of adaptation to different marine environments. This is reflected in morphological differentiation, although not in the traditional shell and girdle characters used to describe polyplacophoran species. The issues raised in this survey indicate that although the nephridiopores and gonopores of lepidopleuran chitons are superficially similar in structure, our current means of identifying and describing their placement is insufficient to consider them to be positionally homologous across taxa for the purpose of systematic inference. The patterns of variance are too clouded by our poor understanding of morphology to shed more light on internal relationships within this group; however, the morphological variation observed here is undoubtedly linked to the high level of evolutionary variance in this diverse group of chitons. Lepidopleurans lack the dramatic colors and patterns of their sister-group the Chitonida, but nonetheless represent a diverse group of marine organisms.

ACKNOWLEDGMENTS

I am grateful to the European Commission SYNTHESIS program for funding research visits to Naturalis

(award NL-TAF-437) and RBINS (award BE-TAF-406). J. Goud and E. Gittenberger provided access to collections in Naturalis (RMNH); J. L. Van Goethem and R. Sablon provided access to collections in RBINS. Thanks also to D. Eernisse for organizing the AMS Chiton Symposium in July 2006 and to the American Malacological Society for financial support for me to attend the symposium. A. N. Hodgson and B. I. Sirenko provided reviews that improved this manuscript.

LITERATURE CITED

- Buckland-Nicks, J. 1995. Ultrastructure of sperm and sperm-egg interaction in Aculifera: Implications for molluscan phylogeny. *Mémoires du Muséum national d'Histoire naturelle, Paris* **166**: 129-153.
- Buckland-Nicks, J. 2006. Fertilization in chitons: Morphological clues to phylogeny. *Venus* **65**: 51-70.
- Dall, W. H. 1889. Report on the results of dredging, under the supervision of Alexander Agassiz, in the Gulf of Mexico (1877-78) and in the Caribbean Sea (1879-80), by the United States Coast Survey Steamer "Blake," Lieutenant-Commander C.D. Sigsbee, U.S.N., and Commander J.R. Bartlett, U.S.N., commanding. Report on the Mollusca, Pt. 2: Gastropoda and Scaphopoda. *Bulletin of the Museum of Comparative Zoology* **18**: 1-492.
- Dell'Angelo, B. and S. Palazzi. 1991. Considerazioni sulla famiglia "Leptochitonidae" Dall 1889 (Mollusca, Polyplacophora). IV. Aggiunte e correzioni. *Bollettino Malacologico* **27**: 35-38 [in Italian].
- Eernisse, D. J. and P. D. Reynolds. 1994. Polyplacophora. In: F. W. Harrison and A. J. Kohn, eds., *Microscopic Anatomy of Invertebrates*, vol. 5: Mollusca I, Wiley-Liss, Inc. New York. Pp. 55-110.
- Hodgson, A. N., J. M. Baxter, M. G. Sturrock, and R. T. F. Bernard. 1988. Comparative spermatology of 11 species of Polyplacophora (Mollusca) from the suborders Lepidopleurina, Chitonina, and Acanthochitonina. *Proceedings of the Royal Society, London (B)* **235**: 161-177.
- Kaas, P. and R. A. Van Belle. 1985. *Monograph of Living Chitons. Vol. 1. Order Neoloricata: Lepidopleurina*. E. J. Brill / Backhuys. Leiden, The Netherlands.
- Kiel, S. and C. T. S. Little. 2006. Cold-seep mollusks are older than the general marine mollusk fauna. *Science* **313**: 1429-1431.
- Okusu, A., E. Schwabe, D. J. Eernisse, and G. Giribet. 2003. Towards a phylogeny of chitons (Mollusca, Polyplacophora) based on combined analysis of five molecular loci. *Organisms, Diversity and Evolution* **3**: 281-302.
- Pelseneer, P. 1899. Sur la morphologie des brachies et des orifices rénaux et génitaux des chitons. *Bulletin scientifique de la France et de la Belgique* **31**: 23 [in French].
- Pilsbry, H. A. 1892. Monograph of the Polyplacophora: Lepidopleuridae. In: G. W. Tryon, eds., *Manual of Conchology*, Academy of Natural Sciences, Philadelphia. Pp. 1-128.
- Plate, L. H. 1899. Die Anatomie und Phylogenie der Chitonen. Fauna Chilensis. *Zoologische Jahrbuch Systematik* 2 (Supplement) **5**: 15-216 [in German].
- Saito, H. and T. Okutani. 1990. Two new chitons (Mollusca: Polyplacophora) from a hydrothermal vent site of the Iheya small ridge, Okinawa Trough, East China Sea. *Venus* **49**: 165-179.
- Sirenko, B. I. 1988. A new genus of deep sea chitons *Ferreiraella* gen. n. (Lepidopleurida, Leptochitonidae) with a description of a new ultra-abyssal species. *Zoological Journal* **67**: 1776-1786.
- Sirenko, B. I. 1993. Revision of the system of the order Chitonida (Mollusca: Polyplacophora) on the basis of correlation between the type of gills arrangement and the shape of the chorion processes. *Ruthenica* **3**: 93-117.
- Sirenko, B. I. 1997. The importance of the development of articulation for taxonomy of chitons (Mollusca, Polyplacophora). *Ruthenica* **7**: 1-24.
- Sirenko, B. I. 2001. Deep-sea chitons (Mollusca, Polyplacophora) from sunken wood off New Caledonia and Vanuatu. *Mémoires du Muséum national d'Histoire naturelle* **185**: 39-71.
- Sirenko, B. I. 2006. New outlook on the system of chitons (Mollusca: Polyplacophora). *Venus* **65**: 27-49.

Submitted: 29 January 2007; **accepted:** 7 August 2007;
final revisions received: 10 February 2008